contrast to the parental compounds were able to bind GM1. The affinity of hybrid CTB for GM1, as estimated by a competitive solid-phase radiobinding assay was unexpectedly high and only 2.5-fold lower than that of its native counterpart. The number of active binding sites on hybrid CTB was determined from: (i) titration with the oligosaccharide moiety of GM1 (oligo-GM1) and monitoring the reversal of the Trp fluorescence quenching by iodide ions and (ii) rapid gel filtration over a superdex HR column of a mixture of hybrid CTB and an excess of 3H-labeled oligo-GM1. The data are in agreement with the formation of one active binding per four reconstituted binding sites in hybrid CTB, which is consistent with a random association of CTB monomers during the denaturation-renaturation cycle.

Tags: Support, Non-U.S. Gov't

Descriptors: *G(M1) Ganglioside--chemistry--CH; *Receptors, Cell Surface --chemistry--CH; Binding Sites; Polymers--chemistry--CH; Succinic Anhydrides; Trypsin

CAS Registry No.: 0 (Polymers); 0 (Receptors, Cell Surface); 0 (Succinic Anhydrides); 0 (choleragen receptor); 108-30-5 (succinic anhydride); 37758-47-7 (G(M1) Ganglioside)

Enzyme No.: EC 3.4.21.4 (Trypsin)

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Interaction of a cholera toxin **derivative containing a reduced number of receptor binding sites with intact cells in culture.**

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Hybrid CTB (hCTB), having only one or two functional binding sites, has been constructed from two chemically inactivated **derivatives** of CTB. One inactive **derivative** consisted of CTB formylated in the lone Trp-88 of each **beta - chain** (fCTB), whereas the other inactive **derivative** consisted of CTB specifically succinylated in three amino groups located in or near the receptor binding site (sssCTB). hCTB, fCTB and sssCTB were able to reassociate with CTA and form the corresponding holotoxins hCT, fCT and sssCT as measured by gel filtration chromatography. In contrast to fCT and sssCT, hCT could increase the cAMP content of intact Vero cells in a time- and dose-dependent way: concentrations as low as a few nanograms of hCT per milliliter caused a significant increase in the intracellular cAMP level. The maximal cAMP level induced by hCT (1 microgram/ml) was, however, more than 2-fold lower than that elicited by its native counterpart. At saturating ligand concentrations and at 37 degrees C, the lag periods and rates of CT and hCT induced cAMP accumulation were essentially the same. Treatment of Vero and HeLa cells with GM1 did not affect their difference in response to CT and hCT. When Vero cells treated with hCT were incubated for longer periods of time, a further slow accumulation of cAMP occurred until after about 20 h cAMP levels of cells exposed to CT or hCT were essentially the same. In contrast to Vero and HeLa cells, human skin fibroblasts exhibited an almost identical response to CT as well as to hCT. Acidotropic agents such as chloroquine and monensin affected the CT and hCT induced increase in cAMP content of Vero cells, fibroblasts and GM1 treated Hela cells in a similar way. The results are consistent with the view that CT receptor recognition domains are shared between adjacent beta-chains, that pentavalent binding appears not to be essential for **cytotoxicity** and that in the cell types studied intracellular processing of CT, hCT is involved.

Tags: Animal; Comparative Study; Human; Support, Non-U.S. Gov't

Descriptors: Cholera **Toxin** --chemistry--CH; *G(M1) Ganglioside --chemistry--CH; *Receptors, Cell Surface--chemistry--CH; Binding Sites;

Protein fold analysis of the B30.2-like domain.

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The B30.2-like domain occurs in some members of a diverse and growing family of proteins containing zinc-binding B-box motifs, whose functions include regulation of cell growth and differentiation. The B30.2-like domain is also found in proteins without the zinc-binding motifs, such as butyrophilin (a **transmembrane** glycoprotein) and stonustoxin (a secreted cytolytic toxin). Currently, the function for the B30.2-like domain is not clear and the structure of a protein containing this domain has not been solved. The secondary structure prediction methods indicate that the B30.2-like domain consists of fifteen or fewer beta-strands. Fold recognition methods identified different structural topologies for the B30.2-like domains. Secondary structure prediction, deletion and lack of local sequence identity at the C-terminal region for certain members of the family, and packing of known core structures suggest that a structure containing two **beta domains** is the most probable of these folds. The most C-terminal sequence motif predicted to be a beta-strand in all B30.2-like domains is a potential subdomain boundary based on the sequence-structure alignments. Models of the B30.2-like domains were built based on immunoglobulin-like folds identified by the fold recognition methods to evaluate the possibility of the B30.2 domain adopting known folds and infer putative functional sites. The SPRY domain has been identified as a subdomain within the B30.2-like domain. If the B30.2-like domain is a subclass of the SPRY domain family, then this analysis would suggest that the SPRY domains are members of the immunoglobulin superfamily.

Tags: Animal; Human
Descriptors: *Models, Molecular; *Protein Folding; *Protein Structure, Secondary; *Proteins--chemistry--CH; Amino Acid Sequence; **Fish Venoms** --chemistry--CH; Membrane Glycoproteins--chemistry--CH; Molecular Sequence Data; Sequence Analysis
CAS Registry No.: 0 (Fish Venoms); 0 (Membrane Glycoproteins); 0 (Proteins); 0 (butyrophilin); 137803-80-6 (stonustoxin)
Record Date Created: 19990628

Structural diversity in a conserved cholera toxin epitope involved in ganglioside binding.

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Cholera is a widespread disease for which there is no efficient vaccine. A better understanding of the conformational rearrangements at the epitope might be very helpful for the development of a good vaccine. Cholera toxin (CT) as well as the closely related heat-labile toxin from Escherichia coli (LT) are composed of two subunits, A and B, which form an oligomeric assembly AB5. Residues 50-64 on the surface of the B subunits comprise a conserved loop (CTP3), which is involved in saccharide binding to the receptor on epithelial cells. This loop exhibits remarkable conformational plasticity induced by environmental constraints. The crystal structure of this loop is compared in the free and receptor-bound toxins as well as in the crystal and solution structures of a complex with TE33, a monoclonal antibody elicited against CTP3. In the toxins this loop forms an irregular structure connecting a beta-strand to the central alpha-helix. Ser 55 and Gln 56 exhibit considerable conformational variability in the five subunits of the unliganded toxins. Saccharide binding induces a change primarily in Ser 55 and Gln 56 to a conformation identical in all five copies. Thus, saccharide binding confers rigidity upon the loop. The conformation of CTP3 in complex with TE33 is quite different. The amino-terminal part of CTP3 forms a beta-turn that fits snugly into a deep binding pocket on TE33, in both the crystal and NMR-derived solution structure. Only 8 and 12 residues out of 15 are seen in the NMR and crystal structures, respectively. Despite these conformational differences, TE33 is cross-reactive with intact CT, albeit with a thousandfold decrease in affinity. This suggests a different interaction of TE33 with intact CT.

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DIPHTHERIA TOXIN PORE FORMATION AND OLIGOMERIZATION IN MEMBRANES: IMPLICATIONS FOR CATALYTIC DOMAIN TRANSLOCATION

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Descriptor Codes: 0487; 0786; 0379

Diphtheria toxin is a cytotoxic protein which has the ability to enter and kill cells by the transfer of its catalytic fragment across cellular endosomes. The mechanism of the translocation of the catalytic fragment is unknown, but it has been suggested that the translocation could occur through a pore. The pores formed by diphtheria toxin have been reported to be as small as 5 A in diameter and as large as to translocate, nor the involvement of the pore in the translocation of the catalytic domain across bilayers. An assay was developed called the dextran leakage assay. This assay involves the trapping of fluorescently labeled dextrans of various sizes inside the lumen of large unilamellar vesicles. The ability of these fluorescently labeled dextrans to escape was detected through the use of antibodies directed against the fluorescent probe attached to the dextran which have the ability to bind to the probe and quench its fluorescence. Using this technique it was found that diphtheria toxin forms concentration dependent pores, that is, at low concentrations toxin pores are small and as the concentration of the toxin in the membrane increases the pore size increases. Toxin oligomerization was found to occur in the membrane and using a combination of chemical crosslinking and rhodamine-self quenching, it was found that the toxin formed non-stoichiometric oligomers. The size of the pores formed by the toxin were affected by addition of cholesterol which increased either the pore number or pore size. In investigating the contribution of the transmembrane domain (T domain) to diphtheria toxin pore formation it was found that the T domain formed concentration dependent pores similar to those of whole toxin but were larger at higher protein concentrations. It was proposed that since the T domain forms larger pores than whole toxin that the catalytic and receptor binding domains of the toxin contributed to the structure of the pore. From these data a mechanism of catalytic domain translocation was proposed in which pore formation was the result of the oligomerization of the toxin in the membrane. The oligomerization may promote the correct membrane orientation of the toxin such that the catalytic domain is correctly positioned for translocation. It was also found that a class of cyclic compounds could inhibit pore formation by diphtheria toxin. Though each of these compounds could inhibit pore formation through steric binding to the channel, inhibition could also occur through several other mechanisms. Continuing studies of the use of these compounds may prove useful in the analysis of other membrane active and pore forming proteins.

Mapping of functional domains within the Saccharomyces cerevisiae type 1 killer preprotoxin.

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Strains of Saccharomyces cerevisiae harboring M1-dsRNA, the determinant of type 1 killer and immunity phenotypes, secrete a dimeric 19-kd toxin that kills sensitive yeast cells by the production of cation-permeable pores in the cytoplasmic membrane. The preprotoxin, an intracellular precursor to toxin, has the domain sequence delta-alpha-gamma-beta where alpha and beta are the 9.5-and 9.0-kd subunits of secreted toxin. Plasmids containing a partial cDNA copy of M1, in which alpha, gamma, and beta are fused to the PH05 promoter and signal peptide, have previously been shown to express phosphate-repressible toxin production and immunity. Here the construction of a complete DNA copy of the preprotoxin gene and its mutagenesis are described. Analysis of the expression of these mutants from the PH05 promoter elucidates the functions of the preprotoxin domains. delta acts as a leader peptide and efficiently mediates the secretion, glycosylation and maturation of killer toxin. Mutations within the **beta subunit** indicate it to be essential for **binding** of **toxin** to and killing of whole cells but unnecessary for the killing of spheroplasts. Mutations within the putative active site of alpha prevent killing of both cells and spheroplasts. The probable role of beta is therefore recognition and binding to the cell wall receptor whereas alpha is the active ionophore. Mutations within alpha causing loss of toxicity also cause loss of immunity, while the mutants described within gamma and beta retain partial or complete immunity. Expression of gamma without alpha or beta confers no phenotype. The immunity determinant may minimally consist of the alpha domain and the N-terminal portion of gamma.(ABSTRACT TRUNCATED AT 250 WORDS)

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Descriptors: *Genes, Fungal; *Genes, Structural; *Mycotoxins--genetics --GE; *Saccharomyces cerevisiae--genetics--GE; Alleles; Base Sequence; Mutation; Phenotype
CAS Registry No.: 0 (Mycotoxins); 0 (killer preprotoxin, yeast)
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